CHAPTER 1

INTRODUCTION

1.1 Introduction

Cyclodextrin glucanotransferase (CGTase, EC 2.4.1.19) is a member of α-amylase family (family 13 of glycosyl hydrolases). Although CGTase is closely related to α-amylase, α-amylase usually catalyze hydrolysis reaction using water as acceptor whereby CGTase preferably catalyze transglycosylation reactions in which glucosyl residues are used as acceptor in forming cyclodextrins (CDs) as the main product. CGTase is a multifunctional enzyme, besides cyclization it also display intermolecular transglycosylation (coupling, disproportionation) and hydrolytic activity on starch and CDs (Nakamura *et al*., 1993).

Currently, bacteria are still regarded as an important source of CGTases. Since the discovery of *Bacillus macerans* as the first source that is capable of producing CGTases, a wide variety of bacteria have been determined as CGTase producers, namely aerobic mesophilic bacteria, aerobic thermophilic, anaerobic thermophilic and aerobic alkalophilic bacteria. Various genera of bacteria that are known as CGTase producer includes *Bacillus*, *Klebsiella*, *Pseudomonas*, *Brevibacterium*, *Thermoanaerobacterium*, *Corynebacterium*, *Micrococcus*, and *Clostridium* (Gawande *et al.,* 1999). Most CGTases produce a mixture of α-, β- and γ-CD in different ratios, depending on the origin of the CGTase as well as the reaction conditions (van der Veen *et al.*, 2000). CGTase is classified into three

different types, α-CGTase, β-CGTase and γ-CGTase according to the major CD produced.

CD molecules have a unique structure with a hydrophobic cavity and hydrophilic outer surface and therefore can form inclusion complexes with a wide variety of hydrophobic guest molecules. Their three dimensional form and size provides an important parameter for complex formation with hydrophobic compounds. Thus, specific (α-, β- and γ-) CD s are required for complexation of specific guest molecules. The formation of inclusion complexes leads to the changes in the chemical and physical properties of the guest molecules. These altered characteristics of encapsulated compounds have led to various applications of CDs in analytical chemistry (Armstrong, 1988; Luong *et al*., 1995), agriculture (Saenger, 1980), biotechnology (Allegre and Deratani, 1994; Szejtli, 1994), pharmacy (Albers and Muller, 1995; Thompson, 1997), food (Allegre and Deratani, 1994; Bicchi *et al*., 1999), and cosmetics (Allegre and Deratani, 1994).

A major disadvantage of CD production by CGTase is that, all known wild type CGTase enzyme produce a mixture α-, β- and γ- CD and are subject to inhibition by these cyclic products (van der Veen *et al.*, 2000). Two different industrial approaches are used to purify the specific produced CD : selective crystallization of β- CD (which is relatively poorly water soluble) and selective complexation with organic solvent. Both of this process makes the production of CD too costly for many applications and the usage of organic solvents limits the application involving human consumption (Pedersen *et al*., 1995). This clearly shows that the availability of CGTase enzymes capable of producing an increased ratio of one particular type of CD and with reduced product inhibition would help to avoid the described expensive and harmful procedures involving organic solvents.

Besides that, for industrial and biochemical studies, it is desirable to develop a novel CGTase that is better in production of CD from starch. This situation has strongly simulated genetic engineering techniques to provide a better CGTase. It was reported that the production of CGTase increased as much as several thousand-fold in protein expression of cloned CGTase protein with the use of genetic

expression promoters (van der Veen *et al.*, 2000). CGTase producing bacteria; alkalophilic *Bacillus* sp. TS1-1 has been successfully isolated by our research group.

The enzyme from this bacterium mainly produces β-CD under the usual reaction condition. Therefore, *Bacillus* sp. TS1-1 can be considered as a good model enzyme for further studies for β-CD production.

1.2 Objective

The objective of this research is to isolate and clone a cyclodextrin glucanotransferase (CGTase) gene from *Bacillus* sp. TS1-1 and to characterize the recombinant enzyme.

1.3 Scope of Research

The scope of this research includes:

a) Bacterial 16S rRNA Identification.
b) Isolation and cloning of CGTase gene from *Bacillus* sp. TS1-1.
c) Sequencing and analysis of the CGTase gene.
d) Expression of CGTase in *E.coli*.
e) To purify the CGTase by using affinity chromatography method.
f) To characterize the purified enzyme.